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(54) Title: MIBEFRADIL ANALOGUES AND THEIR USE

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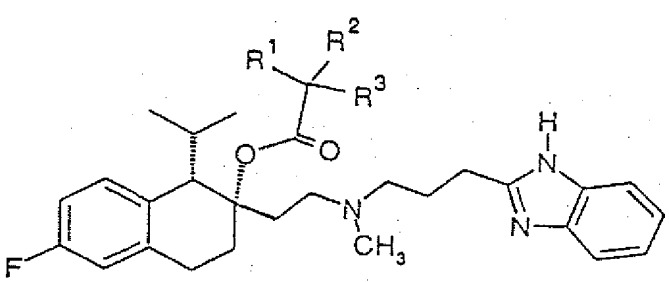


(I)

(57) Abstract: The present invention relates to mibefradil analogues, to compositions comprising the compounds and their use in therapy, e.g. in the treatment and/or prevention of type 1 and type 2 diabetes as well as microvascular or macrovascular diseases associated with diabetes. A compound of formula (I) wherein R^1, R^2 and R^3 independently are H, C_{1-6}-alkyl, C_{3-6}-cycloalkyl, C_{3-6}-cycloalkyl-C_{1-6}-alkyl or C_{1-6}-alkyl-C_{3-6}-cycloalkyl, or a pharmaceutically acceptable salt thereof:

MIBEFRADIL ANALOGUES AND THEIR USE

FIELD OF THE INVENTION

The present invention relates to novel mibefradil analogues, to compositions comprising these compounds and their use in therapy, e.g. in the treatment and/or prevention of type 1 and type 2 diabetes as well as microvascular or macrovascular diseases associated with diabetes.

BACKGROUND OF THE INVENTION

Insulin secretion from pancreatic β-cells is the primary physiological mechanism of blood glucose regulation. A rise in blood glucose concentration stimulates release of insulin from the pancreas, which in turn promotes glucose uptake in peripheral tissues and consequently lowers blood glucose levels, reestablishing euglycemia. Non-insulin dependent diabetes mellitus (NIDDM)(type II diabetes) is associated with impairment in glucose-induced insulin secretion in pancreatic β-cells (Vague, P. and Moulin, J.P., Metabolism 31:139-144 (1982)).

Voltage-gated Ca^{2+} channels mediate a rapidly activated inward movement of Ca^{2+} ions that underlies the stimulation of insulin secretion in β-cells (Boyd, A.E. III, Current Concepts, The Upjohn Company, Kalamazoo, Michigan (1991). In different tissues, four types of Ca^{2+} channels have been described (L(P/Q), T, N, and E channels). The purified L-type Ca^{2+} channel consists of five subunits: α_1, α_2, β, γ, δ (Catterall, W.A., Science 253:1499-1500 (1991)). The primary structure of the α_1 subunit is organized in four homologous domains containing six transmembrane segments (Catterall, W.A., Science 242:50-61 (1988).

Rat and human pancreatic β-cells are equipped with L-type and T-type Ca^{2+} channels (Hiriart, M. and Matteson, D.R., J Gen Physiol 91:145-159 (1988); Davalli, A.M., et al., J Endocrinology 150:195-203 (1996)). L-type Ca^{2+} channels, activated at high voltages and having large unitary conductance and dihydropyridine-sensitivity, are considered the major pipeline for Ca^{2+} influx into the β-cell (Keahey, H.H., et al., Diabetes 38:188-193 (1989)). In contrast, T-type calcium channels activate at low voltages and have small unitary conductance and dihydropyridine-insensitivity.

The physiological function of T-type Ca^{2+} channels in β-cell insulin-secretion has been demonstrated (Bhattacharjee, A., et al., Endocrinology 138:3735-3740 (1997). These channels facilitate exocytosis by enhancing electrical activity in these cells. L-type and T-type Ca^{2+} channels, under normal conditions, work in concert promoting the rise in $[Ca^{2+}]_i$ during glu-

cose-stimulated insulin secretion. In β-cells, over-expressed T-type Ca^{2+} channels may be, at least in part, responsible for the hyper-responsiveness of insulin secretion to non-glucose depolarizing stimuli in GK rat and in rat with NIDDM induced by neonatal injection of streptozotocin (Kato, S., et al., Metabolism 43:1395-1400 (1994); Kato, S., et al., J Clin Invest 97:2417-2425 (1996)). However, over-expressed T-type calcium channels over time will ultimately lead to an elevation of basal Ca^{2+} through it's window current properties. Therefore, there is a dual effect of T-type Ca^{2+} channels in β-cells depending upon channel number and membrane potential.

Two isoforms of L-type Ca^{2+} channel α1 subunits have been identified in β-cells (Seino, S., et al., Proc Natl Acad Sci USA 89:584-588 (1992)). The rat neuronal T-type calcium channel has recently been cloned (Perez-Reyes, E., et al., Nature 391:896-900 (1998)). The α1G subunit of the T-type calcium channel has been cloned from the rat insulin secreting cell line INS-1 (Zhuang et al., *Diabetes* 49: 59-64, 2000). This α1G subunit is expressed in rat islets as well as in brain, neonatal heart and kidney. The α1H subunit of the T-type calcium channel has been cloned from human heart (Cribbs, L.L. *Circ. Res.* 83: 103-109 (1998). Other subunits of T-type Ca^{2+} channel have yet to be identified.

It has recently been described that the blocker of T-type and L-type calcium channels, mibefradil, decrease blood pressure and hyperinsulinemia in fructose fed rats (S. Verma et al, Cardiovascular Research 34: 121-128 (1997)).

In addition it has been shown that there in humans suffering from type 2 diabetes and in animal models of type 2 diabetes is an elevated intracellular level of calcium in both beta-cells and non-pancreatic tissue (J. Levy, Endocrine, 10: 1-6 (1999)) suggesting that compounds which is able to inhibit a rise in intracellular calcium mediated by an influx through T-type calcium channels, can be used to treat or prevent type 2 diabetes and microvascular or macrovascular diseases associated with diabetes.

It is expected that blockers of T-type channels of pancreatic beta cells will protect these cells from the cytotoxic effects of cytokines and will furthermore reduce basal insulin release to reduce the presentations of antibodies associated with Type 1 diabetes. These effects can be used in the treatment on patients suffering from type 1 diabetes as described by Karlsson and Bjork (*Diabetes* 45:1427-30 (1996) and *Autoimmunity* 26:117-122 (1997)).

US 4.808.650 discloses mibefradil and analogues thereof as calcium antagonists which are useful in the treatment of angina pectoris, ischaemia, arrhythmias, high blood pressure and cardiac insufficiency.

The present invention provides a class of novel mibefradil analogues which is able to inhibit a rise in intracellular calcium mediated by an influx through T-type calcium channels, indicating that the compounds of the present invention can be used in the treatment and/or prevention of type 1 and type 2 diabetes as well as microvascular or macrovascular diseases associated with diabetes.

SUMMARY OF THE INVENTION

The present invention relates to novel mibefradil analogues of the general formula (I) wherein R^1, R^2 and R^3 are as defined in the detailed part of the present description.

The present compounds interfere with T-type calcium channel activity and can be used for treating and/or preventing type 1 and type 2 diabetes as well as microvascular or macrovascular diseases associated with diabetes.

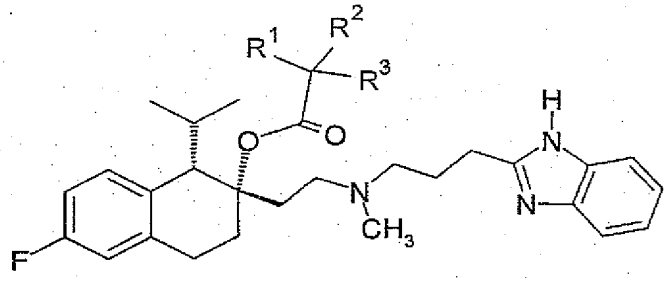
Further, the present compounds are particularly well suited to blocking (inhibiting) the activity of T-type calcium channels but not blocking the activity of L-type calcium channels.

Further provided are pharmaceutical compositions comprising the compounds of the general formula I or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

The invention further provides a method of treating and/or preventing type 1 and type 2 diabetes as well as microvascular or macrovascular diseases associated with diabetes, in a subject (human or animal), the method comprising administering to the subject an amount of a compound effective to modify levels of T-type calcium channels in the pancreatic beta cells of the subject.

DETAILED DESCRIPTION OF THE INVENTION

Accordingly, the present invention relates to novel to mibefradil analogues of the general formula (I)



(I)

wherein R^1, R^2 and R^3 independently are H, C_{1-6}-alkyl, C_{3-6}-cycloalkyl, C_{3-6}-cycloalkyl-C_{1-6}-alkyl or C_{1-6}-alkyl-C_{3-6}-cycloalkyl, or

a pharmaceutically acceptable salt thereof with a pharmaceutically acceptable acid or base.

The present invention also encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other of the present compounds which, upon administration to an animal including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to prodrugs and pharmaceutically acceptable salts of the compounds of the present invention, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents.

In regard to prodrugs, the compounds of the present invention may additionally or alternatively be prepared to be delivered in a prodrug form. The term prodrug indicates a therapeutic agent that is prepared in an inactive form that is converted to an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions.

In regard to pharmaceutically acceptable salts, the term pharmaceutically acceptable salts refers to physiologically and pharmaceutically acceptable salts of the compounds of the present invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. These salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts or optionally alkylated ammonium salts, such as hydrochloric, hydrobromic, hydroiodic, phosphoric, sulfuric, trifluoroacetic, trichloroacetic, oxalic, maleic, pyruvic, malonic, succinic, citric, tartaric, fumaric, mandelic, benzoic, cinnamic, methanesulfonic, ethane sulfonic, picric and the like, and include acids related to the pharmaceutically acceptable salts listed in Journal of Pharmaceutical Science, 66, 2 (1977) and incorporated herein by reference, or lithium, sodium, potassium, magnesium and the like.

The terms "C_{1-6}-alkyl" as used herein, alone or in combination, refers to a straight or branched, saturated hydrocarbon chain having the indicated number of carbon atoms such as e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, 2-methylbutyl, 3-methylbutyl, 4-methylpentyl, n-hexyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 2,2-dimethylpropyl, 1,2,2-trimethylpropyl and the like.

The term "C_{3-6}-cycloalkyl" as used herein refers to a radical of a saturated cyclic hydrocarbon with the indicated number of carbons such as cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

In one embodiment of the invention R^1, R^2 and R^3 are independently H or C_{1-6}-alkyl.

In another embodiment of the invention one of R^1, R^2 and R^3 is H, and the other of R^1, R^2 and R^3 are C_{1-6}-alkyl, e.g. methyl.

In another embodiment of the invention one of R^1, R^2 and R^3 is C_{1-6}-alkyl, e.g. propyl, and the other of R^1, R^2 and R^3 are H.

Specific compounds of the invention are:

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl valeroate;

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl isobutyrate; or
a pharmaceutically acceptable salt thereof.

Other specific compounds of the invention are:

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl isovaleroate;

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl (DL)-2methylbutyrate;

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl cyclopropylacetate;

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl cyclopentylacetate; or

a pharmaceutically acceptable salt thereof.

The present invention is based on the discovery that regulation of T-type calcium channels directly modifies basal calcium levels in cells, which in turn regulates L-type calcium channel activity, which in turn regulates insulin secretion and cell death, which in turn treats e.g. type 2 diabetes. The present invention is further based on the discovery that regulation of T-type calcium channels directly affects basal and glucose-induced insulin secretion. The invention thus provides a method of modifying insulin secretion by pancreatic beta cells, the method comprising modifying levels of T-type calcium channels in the pancreatic beta cells.

Accordingly, in another aspect, the invention relates to pharmaceutical compositions comprising the compounds of the general formula I or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

In another aspect, the invention relates to pharmaceutical compositions for use in the treatment and/or prevention of type 1 and type 2 diabetes as well as microvascular or macrovascular diseases associated with diabetes comprising the compounds of the general formula I or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

In another aspect, the invention relates to the use of a compound of the general formula I or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment and/or prevention of diseases related to the inhibition of a rise in intracellular calcium mediated by an influx through T-type calcium channels.

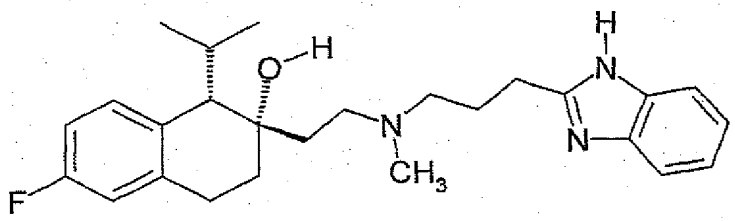
In another aspect, the invention relates to the use of a compound of the general formula I or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment and/or prevention of type 1 and type 2 diabetes as well as microvascular or macrovascular diseases associated with diabetes, such as retinopathy, nephropathy, neuropathy, gangrene, myocardial infarction, cerebral stroke and atherosclerosis.

In another aspect, the invention provides a method of treating and/or preventing type 1 and type 2 diabetes as well as microvascular or macrovascular diseases associated with diabetes, such as retinopathy, nephropathy, neuropathy, gangrene, myocardial infarction, cerebral stroke and atherosclerosis in a subject (human or animal), the method comprising administering to the subject an amount of a compound effective to modify levels of T-type calcium channels in the pancreatic beta cells of the subject.

For therapeutics, methods of modifying insulin secretion by pancreatic beta cells, methods of treating diabetes, methods of modifying basal calcium levels in cells, methods of modifying the action potential of L-type calcium channels in cells, methods of modifying pancreatic beta cell death, methods of modifying pancreatic beta cell proliferation, and methods of modifying calcium influx through L-type calcium channels in cells, each of the methods comprising modifying levels of functional T-type calcium channels in the cells, are provided.

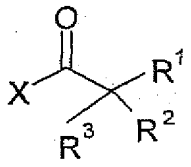
In yet another aspect, the present invention relates to methods of preparing the above-mentioned compounds. The methods comprises:

Reacting a compound of formula (II):



(II)

with an activated carboxylic acid of formula (III):



(III)

wherein R^1, R^2 and R^3 are defined above and X is a leaving group, such as halogen, preferentially chlorine; azide, alkoxy, phenoxy or carbonyloxy. If X is –OH, elevated temperatures and/or a catalyst such as hydrochloric acid will frequently be needed.

The compound of formula (II) may be prepared as described in Y. Crameri et al, *Tetrahedron: Assymetry*, 8: 3617-3623 (1997) and US 4,808,605 or by acid or base catalysed hydrolysis of mibefradil, using standard synthetic procedures as described in e.g. J. March: Advanced Organic Chemistry, 4.ed. 1992, McGraw Hill.

PHARMACOLOGICAL METHODS

Effect of compounds on T-type Ca^{2+} channel α_1G-INS-1 subunit expressed on Xenopus Oocytes or mammalian cells.

Part I. Xenopus Oocyte-two electrode patch clamp recordings

Functional expression of α_1G-INS-1 in Xenopus oocytes.

Oocytes from *Xenopus laevis* will be used for functionally expressing T-type Ca^{2+} channel α1G-INS-1 subunit and for drug screening. Oocytes, at maturation stage V, will be obtained by surgery from anesthetized *Xenopus* frogs. Once removed, the oocytes will be stored in the sterile Barth medium supplemented with penicillin and streptomycin at 19-20°C. The outer vitelline (follicular) layer will be removed either mechanically after osmotic shrinkage in K-aspartate solution or chemically with collagenase/trypsin treatment. Defolliculated oocytes will be again incubated in the Barth medium until injection. Injection will be performed with a pneumatic injector. After microinjection of cRNA, the oocytes will be incubated for three to five days in the antibiotic-supplemented sterile Barth medium at 19-20°C.

Voltage clamp recording and Solutions.

Ca^{2+} currents will be recorded using the conventional two-microelectrode voltage-clamp technique. Voltage recording electrodes will be filled with 3 M KCl and current injecting electrodes with the solution containing (in mM): CsCl 500, EGTA 10, HEPES 10, pH 7.4 (adjusted with CsOH). For the isolation of Ca^{2+} channel current and suppression of the oocyte intrinsic calcium activated Cl^- conductance, Cl^--free methanesulphonate-substituted extracellular solution containing Ba^{2+} as charge carrier will be used (in mM): $Ba(OH)_2$ 40, NaOH 50, KOH 2, HEPES 10, pH 7.4 (adjusted with methanesulphonic acid). To also eliminate Na^+ conductance and maximally suppress K^+, in some cases tetraethylammonium hydroxide will be substituted for NaOH in this solution.

The inhibitory effect of compounds on the T-type Ca^{2+} current will be examined with variable doses. Drugs will be perfused into a chamber where a cell is voltage clamped successfully, T-type Ca^{2+} current will be recorded at 0 mV when held at –90 mV. The designed concentra-

tions will be 10^{-7},10^{-6}, 10^{-5} and 10^{-4} M for each compound. The normalized effect of compounds on current amplitude will be averaged from four or more experiments.

To determine the effect of the compounds on the voltage-dependent properties of the T-type Ca^{2+} channel, the voltage-dependent activation and steady-state inactivation of the T-type Ca^{2+} current expressed in Xenopus oocytes will be characterized. For the voltage dependent activation, the T-type Ca^{2+} current will be recorded at test potentials between –60 mV to +30 mV with increments of 10 mV. For the inactivation, a two second pre-pulse will be applied before a test pulse of 0 mV for 200 mV. Holding potential will be kept at –80 mV for both activation and inactivation characterizations. Normalized conductance-voltage relationship curves were fitted with the Boltzmann equation, $1/\{1+\exp[(V-V_{1/2})/k]\}$, where $V_{1/2}$ is the voltage of half activation and k is a slope factor.

Part II (Alternative). Effect of compounds on the Ca^{2+} currents in HEK-293 cells that expressing α_1G-INS-1 subunit of T-type Ca^{2+} channel.

Permanent expression of α_1G-INS-1 subunit of T-type Ca^{2+} channel in mammalian cells.

Islet isoform of α_1G-INS-1 subunit of T-type Ca^{2+} channel cDNA will be supplied in the pMT2 vertebrate expression vector (Genetics Institute, Cambridge, MA). Green Fluorescent Protein (GFP) cDNA will be excised from Bluescript vector. The GFP fragment will be ligated into pMT2. HEK-293 cells will be transfected by electroporation. 15 µg of pMT2-α1(T) and 1 µg of GFP constructs will be used for transfection. Successfully transfected cells will be identified for electrophysiological recording by expression of GFP.

The whole-cell patch clamp recordings.

The whole-cell recordings will be carried out by the standard "giga-seal" patch clamp technique. The whole-cell recording pipettes will be made of hemocapillaries (Warner Instrument Corp., Hamden, CT), pulled by a two-stage puller (PC-10, Narishige International, New York, NY), and heat polished with a microforge (MF-200, World Precision Instruments, Sarasota, FL) before use. The pipette resistance will be in the range of 2-5 MΩ with our internal solution. The recordings will be performed at room temperature (22°C). Currents were recorded using an EPC-9 patch-clamp amplifier (HEKA, Lambrecht/Pfalz, Germany) and filtered at 2.9 kHz. Data will be acquired with Pulse/PulseFit software (HEKA). Voltage-dependent currents will be corrected for linear leak and residual capacitance by using an on-line P/n subtraction paradigm. Normalized conductance-voltage relationship curves will be fitted with the Boltzmann equation, $1/\{1+\exp[(V-V_{1/2})/k]\}$, where $V_{1/2}$ is the voltage of half activation and k is a slope factor.

Solutions:

Ca^{2+} current recording solution will contain (in mmol/l): 10 $CaCl_2$, 110 tetraethylammonium-Cl (TEA-Cl), 10 CsCl, 10 N -2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 40 sucrose, 0.5 3,4-diaminopyridine, pH 7.3. The pipette solution will contain (in mmol/l): 130 N -methyl-D-glucamine, 20 EGTA (free acid), 5 bis (2-aminophenoxy) ethane-N, N, N', N'-tetraacetate (BAPTA), 10 HEPES, 6 $MgCl_2$, 4 $Ca(OH)_2$, pH was adjusted to 7.4 with methanesulfonate. 2 mmol/l Mg-ATP was included in the pipette solution to minimize run-down of L-type Ca^{2+} currents.

The inhibitory effect of compounds on the T-type Ca^{2+} current will be examined with variable doses. Drugs will be perfused into a chamber where a cell is voltage clamped successfully, T-type Ca^{2+} current will be recorded at 0 mV when held at –90 mV. The designed concentrations will be 10^{-7},10^{-6}, 10^{-5} and 10^{-4} M for each compound. The normalized effect of compounds on current amplitude will be averaged from four or more experiments.

To determine the effect of the compounds on the voltage-dependent properties of the T-type Ca^{2+} channel, we will characterized the voltage-dependent activation and steady-state inactivation of the T-type Ca^{2+} current expressed in HEK cells. For the voltage dependent activation, the T-type Ca^{2+} current will be recorded at test potentials between –60 mV to +30 mV with increments of 10 mV. For the inactivation, a two second pre-pulse will be applied before a test pulse of 0 mV for 200 mV. Holding potential will be kept at –80 mV for both activation and inactivation characterizations. Normalized conductance-voltage relationship curves were fitted with the Boltzmann equation, $1/\{1+\exp[(V-V_{1/2})/k]\}$, where $V_{1/2}$ is the voltage of half activation and k is a slope factor.

2) Effect of compounds on the high voltage activated (e.g. L-type) Ca^{2+} currents in insulin secreting cells.

High voltage activated Ca^{2+} currents will be recorded in INS-1 cells or HIT cells with perforated patch clamp configuration (to prevent L-type Ca^{2+} current "run-up"). In order to eliminate the contamination of T-type Ca^{2+} currents, cell membrane potential will be held at –40 mV and recorded at +20 mV. The time-dependent effect of the compounds on high voltage activated Ca^{2+} current will be examined by sampling the current amplitude every 30 second for 30 minutes after perfusing 10^{-6} M of each compound. If no time-dependent effect is detected, in the next step we will establish the dose-dependent effect of each compound on the high voltage activated Ca^{2+} currents. The designed concentrations will be 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M for each compound. The normalized current amplitude will be averaged from at least four experiments.

The effect of T-type Ca^{2+} modulators can be determined by the measurements of changes in intracellular Ca^{2+} (using microfluorometry and Ca^{2+} sensitive probes such as fluo-3 or fura-2) following a an increase in extracellular K^+ in the presence of the test compound(s).

The cells are kept in an extracellular medium with a slightly reduced K^+ level in order to hyperpolarize the cells and thereby obtain a resting membrane potential which is optimal for the activation of the T-type channel. The stimulatory level of K^+ should be carefully chosen to obtain a depolarization of the cell to a membrane potential where influx through T-type is maximal and at the same time minimizimg influx through other Ca^{2+} channel types. If the cells used contain KATP channels, diazoxide (50-100 microM) may be included in the extracellular media to improve the control of the membrane potential.

Suitable cell lines are INS or RINm5F which both contain T-type Ca^{2+} channels. 5μM ω-conotoxin and 10 μM nifedipine can be added to the incubation medium to block the influx of calcium through the N-type and L-type calcium channels. Cells, which have been transfected with the T-type Ca^{2+} channel, can also be used.

The testing is conducted using the following procedure:

Buffer: Modified KRW (in mM): NaCl 140, KCl 0.5, NaH_2PO_4 0.5, $MgSO_4$ 0.5, $NaHCO_3$ 2, $CaCl_2$ 1.5, HEPES 10, Probenecid 2, pH 7.4.

Protocol: INS-1 cells or BetaTC3 cells were cultured in black-walled 96-well plates (Packard View-Plate) under normal conditions. They were washed and loaded in modified KRW, 1 mM D-glucose to repolarise the cells, with the fluorescent calcium indicator Fluo-4/AM (1 μM) in the presence of 2 mM Probenecid for 30 min. After washing in the same modified KRW and addition of modified KRW, supplemented or not with 10 μM Nifedipine and/or 50 μM BPDZ 73, the cell plate was placed in the FLIPR. Automated addition of a KCl gradient was done in separate experiments after which 10 and 30 mM KCl, giving about 50 and 100% response, were chosen as fixed concentrations for successive studies of the test compounds. The compounds were tested as 10 point 1:3 dilution series, with 50 μM as the highest concentration. The changes in Fluo-4 fluorescence were followed every two or six seconds for 3-10 min during compound addition and the addition of KCl (two different protocols, thereby the variance in timing). A Katp channel opener, BPDZ 73, at 10 μM was added to ensure full repolarisation the cells, dependent on K_{ATP} channels.

PHARMACEUTICAL COMPOSITIONS

The formulation of pharmaceutical compositions and their subsequent administration is believed to be within the skill in the art. In general, for therapeutics, a patient suspected of needing such therapy is given a composition in accordance with the invention, commonly in a pharmaceutically acceptable carrier, in amounts and for periods which will vary depending upon the nature of the particular disease, its severity and the patient's overall condition. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral or parenteral. Parenteral administration includes intravenous drip or infusion, subcutaneous, intraperitoneal or intramuscular injection, pulmonary administration, e.g., by inhalation or insufflation, or intrathecal or intraventricular administration.

Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

Compositions for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions, which may also contain buffers, diluents and other suitable additives.

In addition to such pharmaceutical carriers, cationic lipids may be included in the formulation to facilitate uptake. One such composition shown to facilitate uptake is LIPOFECTIN (BRL, Bethesda MD).

Dosing is dependent on severity and responsiveness of the condition to be treated, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual compositions, and can generally be calculated based on IC_{50}'s or EC_{50}'s in in vitro and in vivo animal studies. For example, given

the molecular weight of compound (derived from chemical structure) and an effective dose such as an IC_{50}, for example (derived experimentally), a dose in mg/kg is routinely calculated.

The compounds of the invention may be administered to a mammal, especially a human, in need of treatment prevention, elimination alleviation or amelioration of the diseases as mentioned above. Such mammals include also animals, both domestic animals and non-domestic animals.

Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

EXAMPLES

The process of preparing the compounds of formula I is further illustrated in the following examples which, however, are not to be construed as limiting.

EXAMPLE 1

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl valeroate

2-(2-{[3-(1-Benzoimidazol-2-yl)-propyl]-methyl-amino}-ethyl)-6-fluoro-1-isopropyl-1,2,3,4-tetrahydro-2-naphthalinol

Methoxyacetic acid 2(S)-[2-[N-[3-(2-benzimidazolyl)propyl]-N-methylamino]ethyl]-6-fluoro-1(S)-isopropyl-1,2,3,4-tetrahydro-2-naphthyl ester dihydrochloride (Mibefradil, 0.570g) in ethanol (96%, 5 ml) and aqueous sodium hydroxide (1 N, 5 ml) was refluxed for 2 h. The cold reaction mixture was concentrated. The residue was partitioned between water and dichloromethane. The aqueous layer was extracted with dichloromethane (2X). The combined organic layers were dried (sodium sulfate) and concentrated to give the title compound as a clear syrup 0.43g (100%).

1H-NMR ($CDCl_3$): δ 7.57 (broad, 2H); 7.23 (m, 2H); 6.97 (m, 1H); 6.58 (m, 2H); 3.07-2.83 (m, 3H); 2.75 (m, 1H); 2.6 (m, 4H); 2.5-2.2 (s + m, 3H +3H); 2.06 (p, 2H); 1.81 (broad dd, 1H); 1.50 (m, 2H); 1.20 (d, 3H); 0.53 ppm (d, 3H).

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl valeroate dihydrochloride

2-(2-{[3-(1-Benzoimidazol-2-yl)-propyl]-methyl-amino}-ethyl)-6-fluoro-1-isopropyl-1,2,3,4-tetrahydro-2-naphthalinol (0.080g) was dissolved in dichloromethane (2 ml). Diisopropylethylamine (0.033ml) and valeroylchloride (0.070ml) was added. After stirring for 70 h, aqueous saturated sodium hydrogencarbonate was added. The aqueous layer was extracted with dichloromethane (2X). The combined organic layers were dried (sodium sulfate) and concentrated. The residue was purified by flash chromatography using dichloromethane/methanol 6:1 as eluent to give the free base as a sirup (0.070g, 84%). This product was dissolved in ethanol and aqueous hydrochloride (1 N, 0.38 ml) was added. After stirring for 30 min the mixture was concentrated. The residue was crystallized from ethyl acetate to give the title compound as a white powder (30 mg, 27%).
Mp 118-121°C

EI SP/MS: 507 (M+)

1H-NMR (DMSO): δ 7.75 (m, 2H); 7.49 (m, 2H); 7.08 (m, 1H); 6.96 (broad d, 2H); 3.15 (m, 4H); 2.95 (m, 3H); 2.7 (s, 3H); 2.48 (dt, 2H); 2.23 (m, 2H); 2.0 (m, 4H); 1.50 (p, 2H); 1.35 (p, 2H); 1.02 (d, 3H); 0.90 (t, 3H); 0.35 ppm (d, 3H).

EXAMPLE 2

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl isobutyrate dihydrochloride

2-(2-{[3-(1-Benzoimidazol-2-yl)-propyl]-methyl-amino}-ethyl)-6-fluoro-1-isopropyl-1,2,3,4-tetrahydro-2-naphthalinol (0.110g) was dissolved in dichloromethane (1 ml). Diisopropylethylamine (0.082ml) and isobutyryl chloride (0.082ml) was added. After stirring for 19 h

the reaction mixture was worked up and purified as described in EXAMPLE 1 to give the title compound (58 mg, 39%)

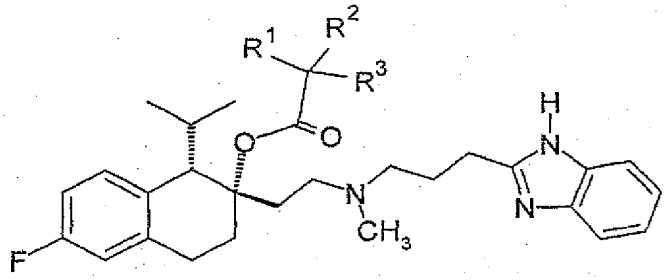
Mp 114-117°C

EI SP/MS: 493 (M+)

1H-NMR (DMSO): δ 7.77 (n, 2H); 7.52 (m, 2H); 7.07 (m, 1H); 6.96 (broad d, 2H); 3.43 (m, 1H); 3.3- 3.05 (m, 5H); 2.97 (m, 2H); 2.88 (m, 1H); 2.70 (s, 3H); 2.61 (m, 1H); 2.45 (m, 1H); 2.3 (m, 2H);2.15-1.85 (m, 4H); 1.15 (d, 6H); 1.00 (d, 3H); 0.35 ppm (d, 3H).

CLAIMS

1. A compound of formula I



(I)

wherein R^1, R^2 and R^3 independently are H, C_{1-6}-alkyl, C_{3-6}-cycloalkyl, C_{3-6}-cycloalkyl-C_{1-6}-alkyl or C_{1-6}-alkyl-C_{3-6}-cycloalkyl, or
a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein R^1, R^2 and R^3 independently are H or C_{1-6}-alkyl.

3. A compound according to claim 1 or 2 wherein one of R^1, R^2 and R^3 is H, and the other of R^1, R^2 and R^3 are C_{1-6}-alkyl.

4. A compound according to claim 3 wherein one of R^1, R^2 and R^3 is H, and the other of R^1, R^2 and R^3 are methyl.

5. A compound according to claim 1 or 2 wherein one of R^1, R^2 and R^3 is C_{1-6}-alkyl, and the other of R^1, R^2 and R^3 are H.

6. A compound according to claim 5 wherein one of R^1, R^2 and R^3 is butyl, and the other of R^1, R^2 and R^3 are H.

7. A compound according to any of the preceding claims selected from the following:

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl valeroate;

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl isobutyrate; or

a pharmaceutically acceptable salt thereof.

8. A compound according to any of the preceding claims selected from the following:

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl isovaleroate;

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl (DL)-2methylbutyrate;

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl cyclopropylacetate;

(1S,2S)-2-(2-{N-[(3-benzoimidazol-2-yl)propyl]-N-methylamino}ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl cyclopentylacetate; or

a pharmaceutically acceptable salt thereof.

9. A pharmaceutical composition comprising a compound according to any of the preceding claims or a pharmaceutical acceptable salt thereof together with one or more pharmaceutically acceptable carriers or diluents.

10. A pharmaceutical composition for use in the treatment and/or prevention of type 2 diabetes comprising a compound according to any of the preceding claims 1-8 or a pharmaceutical acceptable salt thereof together with one or more pharmaceutically acceptable carriers or diluents.

11. A pharmaceutical composition for use in the treatment and/or prevention of type 1 diabetes comprising a compound according to any of the preceding claims 1-8 or a pharmaceutical acceptable salt thereof together with one or more pharmaceutically acceptable carriers or diluents.

12. A pharmaceutical composition for use in the treatment and/or prevention of microvascular or macrovascular diseases associated with diabetes comprising a compound according to any of the preceding claims 1-8 or a pharmaceutical acceptable salt thereof together with one or more pharmaceutically acceptable carriers or diluents.

13. The pharmaceutical composition according to any of the claims 9-12 in the form of an oral dosage unit or parenteral dosage unit.

14. The use of a compound according to any one of the claims 1 – 8 or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment and/or prevention of disorders related to the inhibition of a rise in intracellular calcium mediated by an influx through T-type calcium channels.

15. The use of a compound according to any one of the claims 1 - 8 or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment and/or prevention of type 2 diabetes.

16. The use of a compound according to any one of the claims 1 - 8 or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment and/or prevention of type 1 diabetes.

17. The use of a compound according to any one of the claims 1 - 8 or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment and/or prevention of microvascular or macrovascular diseases associated with diabetes.

18. The use of a compound according to any one of the claims 1 - 8 or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment and/or prevention of retinopathy.

19. The use of a compound according to any one of the claims 1 - 8 or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment and/or prevention of nephropathy.

20. The use of a compound according to any one of the claims 1 - 8 or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment and/or prevention of neuropathy.

21. The use of a compound according to any one of the claims 1 - 8 or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment and/or prevention of macrovascular diseases associated with gangrene, myocardial infarction, cerebral stroke or atherosclerosis.

22. A method of treating and/or preventing of disorders related to the inhibition of a rise in intracellular calcium mediated by an influx through T-type calcium channels in a subject in need thereof comprising administering an effective amount of a compound according to any of the claims 1 - 8 to said subject.

23. A method of treating and/or preventing type 2 diabetes in a subject in need thereof comprising administering an effective amount of a compound according to any of the claims 1 - 8 to said subject.

24. A method of treating and/or preventing type 1 diabetes in a subject in need thereof comprising administering an effective amount of a compound according to any of the claims 1 - 8 to said subject.

25. A method of treating and/or preventing microvascular or macrovascular diseases associated with diabetes in a subject in need thereof comprising administering an effective amount of a compound according to any of the claims 1 - 8 to said subject.

26. A method of treating and/or preventing retinopathy in a subject in need thereof comprising administering an effective amount of a compound according to any of the claims 1 - 8 to said subject.

27. A method of treating and/or preventing nephropathy in a subject in need thereof comprising administering an effective amount of a compound according to any of the claims 1 - 8 to said subject.

28. A method of treating and/or preventing neruopathy a subject in need thereof comprising administering an effective amount of a compound according to any of the claims 1 - 8 to said subject.

29. A method of treating and/or preventing macrovascular diseases associated with gangrene, myocardial infarction, cerebral stroke or atherosclerosis in a subject in need thereof comprising administering an effective amount of a compound according to any of the claims 1 - 8 to said subject.

30. A process for the manufacture of a pharmaceutical composition, particular to be used in the treatment and/or prevention of type 2 diabetes, which process comprising bringing a compound of formula I according to any of the claims 1 - 8 or a pharmaceutically acceptable salt thereof into a galenic dosage form.

31. A process for the manufacture of a pharmaceutical composition, particular to be used in the treatment and/or prevention of type 1 diabetes, which process comprising bringing a compound of formula I according to any of the claims 1 - 8 or a pharmaceutically acceptable salt thereof into a galenic dosage form.

32. A process for the manufacture of a pharmaceutical composition, particular to be used in the treatment and/or prevention of microvascular or macrovascular diseases associated with diabetes, which process comprising bringing a compound of formula I according to any of the claims 1 - 8 or a pharmaceutically acceptable salt thereof into a galenic dosage form.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 01/00128

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07D 235/14, A61K 31/4184, A61P 5/48, A61P 9/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07D, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Cardiovascular Research, Volume 34, 1997, Subodh Verma et al, "Chronic T-type Ca 2+ channel blockade with mibefradil in hyperinsulinemic, insulin-resistant and hypertensive rats" page 121 - page 128 --	1-32
X	Cardiovascular Research, Volume 39, 1998, Steffen Sandmann et al, "Effects of the calcium channel antagonist mibefradil on haemodynamic and morphological parameters in myocardial infarction-induced cardiac failure in rats" page 339 - page 350 --	1-14,21-22, 29-32
X	US 4808605 A (BRANCA ET AL), 28 February 1989 (28.02.89), the claims --	1-14,21-22, 29-32

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 June 2001	27 -06- 2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Solveig Gustavsson/BS Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 01/00128

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 0015845 A1 (SOUTH ALABAMA MEDICAL SCIENCE FOUNDATION), 23 March 2000 (23.03.00), page 38 - page 42	1-32
	--	
A	WO 0002543 A1 (NOVARTIS-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H.), 20 January 2000 (20.01.00)	1-32
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK01/00128

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 22-29
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Claims 22-29 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/05/01

International application No.
PCT/DK 01/00128

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4808605 A	28/02/89	AT 70267 T	15/12/91
		AU 600769 B	23/08/90
		AU 8090987 A	19/05/88
		BG 60937 B	28/06/96
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		CS 264350 B	12/07/89
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		CS 9103948 A	17/06/92
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		EP 0268148 A,B	25/05/88
		SE 0268148 T3	
		ES 2040234 T	16/10/93
		FI 94414 B,C	31/05/95
		FI 875024 A	15/05/88
		GR 3004029 T	31/03/93
		HK 110494 A	21/10/94
		HU 215915 B	29/03/99
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		JP 2504490 B	05/06/96
		JP 63139171 A	10/06/88
		KR 9604863 B	16/04/96
		LV 5745 A,B	20/10/96
		MC 1883 A	24/01/89
		MX 9271 A	01/09/93
		MX 173614 B	18/03/94
		NO 172237 B,C	15/03/93
		NO 874757 A	16/05/88
		NZ 222474 A	26/02/90
		PH 23919 A	23/01/90
		PT 86130 A,B	01/12/87
		SG 114194 G	25/11/94
		ZA 8708362 A	16/05/88
		ZW 20087 A	20/07/88
WO 0015845 A1	23/03/00	AU 6021799 A	03/04/00
		EP 0963215 A	15/12/99
WO 0002543 A1	20/01/00	AU 5034999 A	01/02/00
		BR 9912021 A	03/04/01
		EP 1096932 A	09/05/01
		NO 20010113 A	09/03/01